Untenospongin C, a New C_{21} Furanoterpene from the Okinawan Marine Sponge *Hippospongia* Sp.

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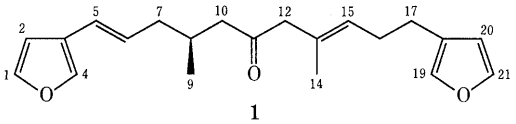
A new C_{21} furanoterpene, untenospongin C (1), has been isolated from the Okinawan marine sponge *Hippospongia* sp. and its structure was determined on the basis of the spectroscopic data and chemical evidence. The absolute configuration of untenospongin B (2) was established by an examination of the NMR data for the 2-methoxy-2-trifluoromethylphenylacetic acid esters of 2.

Keywords untenospongin C; *Hippospongia* sp.; sponge; furanoterpene; 2-methoxy-2-trifluoromethylphenylacetic acid

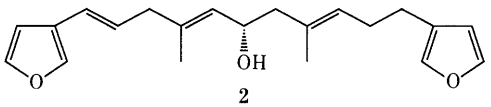
Marine sponges of the genus *Hippospongia* are a rich source of bioactive compounds such as sesquiterpenoids[1] and furanoterpenes.[2] During our studies on bioactive substances from marine organisms,[3] we investigated extracts of the Okinawan sponge *Hippospongia* sp. and isolated a new C_{21} furanoterpene, named untenospongin C (**1**), together with a known related compound, untenospongin B (**2**).[4] Hence we describe the isolation and structure elucidation of **1** and determination of the absolute configuration of **2**.

The sponge *Hippospongia* sp. was collected at Unten Harbor, Okinawa Island, and kept frozen until used. MeOH extract of the sponge was partitioned between EtOAc and H_2O. The EtOAc-soluble fraction was subjected to silica gel column chromatography eluted with hexane–EtOAc (4:1) followed by preparative TLC (hexane–diethyl ether, 8:1) and reversed-phase HPLC (MeOH–H_2O, 95:5) to afford untenospongin C (**1**, 0.001%, wet weight) together with untenospongin B (**2**, 0.03%).

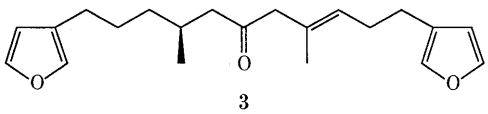
The high resolution electron impact MS (HREIMS) data of untenospongin C (**1**) established the molecular formula, $C_{21}H_{26}O_3$ (*m/z* 326.1910, M^+, Δ +2.8 mmu), which was supported by the ^{13}C-NMR spectrum showing 21 carbon signals. The IR spectrum of **1** indicated the presence of a ketone carbonyl group (ν_{max} 1710 cm^{-1}). The ^{1}H-NMR spectrum of **1** showed the presence of an *E*-disubstituted double bond (δ_H 6.21, d, J=15.6 Hz, δ_H 5.86, dt, J=15.6, 7.3 Hz) and one *E*-trisubstituted double bond (δ_H 5.26, t, J=6.6 Hz) bearing a methyl group (δ_H 1.59, s; δ_C 16.5, q). The ^{1}H-and ^{13}C-NMR spectra of **1** were similar to those of untenospongin B (**2**), suggesting that **1** is a C_{21} furanoterpene having two furan rings (δ_H 6.27, 6.49, 7.21, and 7.34; δ_C 107.6, 111.0, 124.3, 124.6, 138.9, 139.6, 142.7, and 143.3).[5] The ^{1}H–^{1}H correlation spectroscopy (COSY) spectrum of **1** revealed the presence of four segments, C-1 to C-4, C-5 to C-7, C-12 to C-17, and C-19 to C-21, which were also found in untenospongin B (**2**). The structural difference between compounds **1** and **2** was found in the C-8—C-11 segment. The methylene protons (δ_H 2.42, H_2-10) adjacent to a carbonyl group (δ_C 209.2, C-11) were coupled to one methine proton (δ_H 2.20, H-8) on carbon bearing a methyl group (δ_H 0.92, H_3-9) observed in **1** in place of signals due to an olefinic proton (δ_H 5.23, H-10) and a vinyl methyl group (δ_H 1.70, H_3-9) in **2**.



1



2

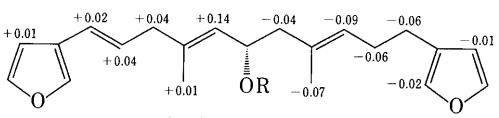


3

Catalytic reduction of **1** with Pd–C under a hydrogen atmosphere afforded compound **3**, corresponding to a reductive product of the C-5 double bond of **1**. The optical rotation and other spectral data of **3** were completely consistent with those of dihydrofurospongin-2.[6] So, the absolute configuration at C-8 of **1** was assigned as *S*, which is the same as that of dihydrofurospongin-2.[6] Thus, the structure of untenospongin C was concluded to be **1**.

In order to determine the absolute configuration of untenospongin B (**2**), **2** was converted into the *S*- and *R*-2-methoxy-2-trifluoromethylphenylacetic acid (MTPA) esters (**4** and **5**, respectively). The values of $\Delta\delta$ [δ(*S*-MTPA ester)-δ(*R*-MTPA ester)] observed for H-5, H-6, H-7, H-10 were +0.02, +0.04, +0.04, and +0.14 ppm, while those observed for H-12, H-14, H-15, H-16 were −0.04, −0.09, −0.06, and −0.06 ppm, respectively (Fig. 1). These results suggested that the absolute configuration at C-11 of **2** is *S*.[7]

Untenospongin C (**1**) is a new C_{21} furanoterpene from the sponge *Hippospongia* sp. Such C_{21} furanoterpenes have also been isolated from sponges of the genera *Spongia*[8] and *Carteriospongia*.[9] Untenospongin C (**1**) exhibited cytotoxicity against murine lymphoma L1210 cell *in vitro* with the IC_{50} value of 3.8 μg/ml.



4: R=(*S*)-MTPA
5: R=(*R*)-MTPA

Fig. 1. ^{1}H-NMR Chemical Shift Differences ($\Delta\delta$) for MTPA Esters of Untenospongin B (**2**)

$\Delta\delta$ (ppm) = δ[(*S*)-MTPA ester] − δ[(*R*)-MTPA ester].

Experimental

General Methods Optical rotations were recorded on a JASCO DIP-370 digital polarimeter. UV and IR spectra were taken on a Shimadzu UV-220 spectrometer and a JASCO Report-100 infrared spectrometer, respectivey. ^{1}H- and ^{13}C-NMR spectra were recorded on JEOL JMN GX-270 and EX-400 spectrometers in $CDCl_3$. The residual chloroform resonances at δ_H 7.26 and δ_C 77.1 were used as internal references for ^{1}H- and ^{13}C-NMR spectra, respectively. EIMS were obtained on a JEOL JMS DX-303 spectrometer operating at 70 eV.

Collection, Extraction, and Isolation The sponge *Hippospongia* sp. was collected at Unten Harbor, Okinawa Island, and kept frozen until used. The sponge (0.9 kg, wet weight) was extracted with MeOH (1 l × 2) and then evaporated to give a residue (26.6 g). The residue was partitioned between ethyl acetate (400 ml × 3) and 1 M NaCl (400 ml). The ethyl acetate-soluble portion was evaporated under reduced pressure to give a residue (1.97 g), which was subjected to silica gel column chromatography (Wako gel C-300, Wako Pure Chemical, 2.3 × 40 cm) with hexane–EtOAc [4 : 1 (800 ml)→3 : 1(300 ml)→2 : 1(300 ml)]. The fraction eluting from 120 to 140 ml was subjected to preparative TLC (Merck, Kiesel gel 60, F_{254}) with hexane–diethyl ether (8 : 1 × 2), followed by purification by reversed-phase HPLC (YMC-Pack AM-323 ODS, YMC Co., 10 × 250 mm; flow rate, 2.5 ml/min; UV detection at 254 nm; MeOH–H_2O 95 : 5) to afford untenospongin C (**1**, 9.2 mg, 0.001% wet weight, t_R 8.0 min). The fraction eluting at 210 to 290 ml from the first silica gel column was subjected to preparative TLC with hexane–EtOAc (5 : 1 × 3) and preparative HPTLC with hexane–ethyl acetate (2 : 1), followed by Sep-Pak Silica (Waters) with hexane (10 ml) and then hexane–EtOAc (8 : 1) to give untenospongin B (**2**, 260 mg, 0.03%) in the latter fraction.

Untenospongin C (1) A colorless oil, $[\alpha]_D^{20}$ −9.3° (c = 1.0, $CHCl_3$). IR ν_{max}^{neat}: 1710 cm^{-1}: UV λ_{max}^{EtOH} nm (ε): 212 (23600), 222 (sh). ^{1}H-NMR ($CDCl_3$) δ_H: 0.92 (3H, d, J = 6.6 Hz, H_3-9), 1.59 (3H, s, H_3-14), 2.07 (2H, dt, J = 11.7, 5.9 Hz, H_2-7), 2.20 (1H, m, H-8), 2.22 (1H, dd, J = 16.0, 7.1 Hz, H′-10), 2.29 (2H, dt, J = 7.3, 6.3 Hz, H_2-16), 2.42 (1H, dd, J = 16.0, 5.4 Hz, H-10), 2.46 (2H, t, J = 7.3 Hz, H_2-17), 3.01 (2H, s, H_2-12), 5.26 (1H, t, J = 6.6 Hz, H-15), 5.86 (1H, dt, J = 15.6, 7.3 Hz, H-6), 6.21 (1H, d, J = 15.6 Hz, H-5), 6.27 (1H, s, H-20), 6.49 (1H, s, H-2), 7.21 (1H, s, H-19), 7.34 (3H, s, H-1, H-4 and H-21). ^{13}C-NMR ($CDCl_3$) δ_C: 16.5 (q, C-14), 19.3 (q, C-9), 24.7 (t, C-17), 28.5 (t, C-16), 29.3 (d, C-8), 40.2 (t, C-7), 48.2 (t, C-10), 54.5 (t, C-12), 107.6 (d, C-2), 111.0 (d, C-20), 121.2 (d, C-5), 124.3 (s, C-3), 124.6 (s, C-18), 128.3 (d, C-6), 129.0 (d, C-15), 129.7 (s, C-13), 138.9 (d, C-19), 139.6 (d, C-4), 142.7 (d, C-21), 143.3 (d, C-1), 209.2 (s, C-11). ^{1}H–^{1}H COSY correlations ($CDCl_3$, H/H): H-1/H-2, H-5/H-6, H-6/H-7, H-7/H-8, H-8/H-9, H-8/H-10, H-15/H-16, H-16/H-17, H-20/H-21. EIMS m/z: 326 (M^+). HREIMS m/z: 326.1910 (M^+, Calcd for $C_{21}H_{26}O_3$: 326.1882).

Reduction of Untenospongin C (1) Pd–C (10%, 2.0 mg) was added to an ethyl acetate solution (1.5 ml) of untenospongin C (**1**, 2.3 mg). The reaction mixture was stirred under an H_2 atmosphere at room temperature for 2 h. Pd–C was removed by filtration, and evaporation of the solvent afforded 5,6-dihydrountenospongin C (**3**, 2.2 mg, 96%), a colorless oil, $[\alpha]_D^{21}$ −6.4° (c = 0.37, $CHCl_3$). IR ν_{max}^{neat}: 1710 cm^{-1}. UV λ_{max}^{hexane} nm (ε): 211 11100). ^{1}H-NMR ($CDCl_3$) δ: 0.87 (3H, d, J = 6.6 Hz, H-9), 1.58 (3H, s, H-14), 2.02—2.04 and 2.12—2.50 (2H and 11H, respectively, m, H-5, H-6, H-7, H-8, H-10, H-16, and H-17), 3.01 (2H, s, H-12), 5.27 (1H, t, H-15), 6.26 (1H, s, H-20), 6.28 (1H, s, H-2), 7.20 (2H, s, H-4 and H-19), 7.34 (2H, s, H-1 and H-21). EIMS m/z: 328 (M^+). HREIMS m/z: 328.2023 (M^+, Calcd for $C_{21}H_{28}O_3$: 328.2039).

(*S*)-MTPA Ester (4) of Untenospongin B (*S*)-MTPA chloride (25.0 mg, 99 μmol) was added to a solution of untenospongin B (**2**, 11.6 mg, 36 μmol) in anhydrous pyridine (1 ml), and the solution was allowed to stand at room temperature for 15 h. 3-[(Dimethylamino)propyl]amine (16.5 mg, 161 μmol) was added and after 10 min the solvent was evaporated off. The residue was subjected to preparative TLC [hexane–EtOAc (10 : 1)] to give the (*S*)-MTPA ester (**4**, 14.2 mg) of untenospongin B. ^{1}H-NMR ($CDCl_3$) δ: 1.57 (3H, s, H_3-14), 1.79 (3H, s, H_3-9), 2.16 (2H, dt, J = 7.8, 6.8 Hz, H_2-16), 2.36 (2H, d, J = 7.8 Hz, H_2-12), 2.40 (2H, t, J = 7.8 Hz, H_2-17), 2.84 (2H, d, J = 6.8 Hz, H_2-7), 3.51 (3H, s, MeO), 5.16 (1H, t, J = 6.8 Hz, H-15), 5.24 (1H, d, J = 9.3 Hz, H-10), 5.84 (1H, dt, J = 15.6, 6.8 Hz, H-6), 5.88 (1H, dd, J = 9.3, 7.8 Hz, H-11), 6.23 (1H, s, H-20), 6.24 (1H, d, J = 15.6 Hz, H-5), 6.47 (1H, s, H-2), 7.16 (1H, s, H-19), 7.33—7.51 (8H, m, H-1, H-4, H-21 and Ph). EIMS m/z: 542 (M^+).

(*R*)-MTPA Ester (5) of Untenospongin B (*R*)-MTPA chloride (37.4 mg, 148 μmol) was added to a solution of untenospongin B (**2**, 11.0 mg, 34 μmol) in anhydrous pyridine (1 ml), and the solution was allowed to stand at room temperature for 15 h. 3-[(Dimethylamino)propyl]amine (26.3 mg, 258 μmol) was added, and after 10 min, the solvent was evaporated off. The residue was subjected to preparative TLC [hexane–EtOAc (10 : 1)] to afford the (*R*)-MTPA ester (**5**, 14.2 mg, 78%) of untenospongin B. ^{1}H-NMR ($CDCl_3$) δ: 1.64 (3H, s, H_3-14), 1.78 (3H, s, H_3-9), 2.22 (2H, dt, J = 8.5, 6.6 Hz, H_2-16), 2.40 (2H, d, J = 7.3 Hz, H_2-12), 2.46 (2H, t, J = 8.5 Hz, H_2-17), 2.80 (2H, d, J = 6.8 Hz, H_2-7), 3.52 (3H, s, MeO), 5.10 (1H, d, J = 9.3 Hz, H-10), 5.25 (1H, br t, J = 6.6 Hz, H-15), 5.80 (1H, dt, J = 15.6, 6.8 Hz, H-6), 5.85 (1H, dd, J = 9.3, 7.3 Hz, H-11), 6.22 (1H, d, J = 15.6 Hz, H-5), 6.24 (1H, s, H-20), 6.46 (1H, s, H-2), 7.18 (1H, s, H-19), 7.33—7.48 (8H, m, H-1, H-4, H-21 and Ph). EIMS m/z: 542 (M^+).

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